

Gender modulates the energy cost of muscle contraction in untrained healthy subjects. A ^{31}P magnetic resonance spectroscopy analysis

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Abstract The forearm flexor muscles of 56 untrained volunteers (26 women and 30 men) were examined by ^{31}P magnetic resonance spectroscopy, during a rest-exercise-recovery protocol, in order to document the impact of gender on muscle energetics. Absolute concentrations of high-energy phosphate compounds, intracellular pH and rates of aerobic and anaerobic ATP production were calculated. An inverse correlation was found between body mass index (BMI) and power output in women but not in men. After correcting for power output and BMI, the measured energy cost of contraction was twice larger for women than for men. This increase was also reflected in larger ATP production from aerobic and anaerobic pathways. This higher energy cost might be explained in part by differences in local muscle mass, a higher impact of fatness, but also by a reduced metabolic efficiency of muscle fibers in untrained women.

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Key words: Muscle metabolism; Gender; Age;

^{31}P Magnetic resonance spectroscopy

1. Introduction

It is commonly accepted that males display greater strength and muscle capacity than females [1,2], even late in life [3–6]. Although these differences can be explained partly in terms of muscle cross-sectional area [2] and training backgrounds, the underlying metabolic causes still remain obscure.

A larger aerobic capacity has been shown [1] during contraction in males as compared to females, with quantitative differences in fiber distribution. In contradiction, a larger anaerobic capacity without quantitative differences in muscle fiber composition has also been reported [7]. It has been suggested that women could be better suited than men to prolonged moderate exercise [8] possibly as a result of increased fat utilization delaying depletion of glycogen stores [9,10]. Studies of enzyme activities on biopsy samples have indicated that the relative contribution of oxidative metabolism was similar [1,11] or higher in women [12], whereas the glycolytic capacity was more pronounced in men [1,7,13].

Due to its non-invasive nature and high informational content, ^{31}P magnetic resonance spectroscopy (MRS) is widely and increasingly used to study energetics in normal and diseased muscles. However, no study has been devoted so far to the analysis of gender impact per se on muscle energetics.

The aim of this work was to address the impact of gender,

and secondarily of age, on muscle energetics through a quantitative ^{31}P MRS analysis.

2. Patients and methods

Fifty-six untrained subjects, 26 women and 30 men, volunteered to participate in this study, which was approved by the Committee on Ethics. Twenty-seven subjects were over the age of 50 years and were classified arbitrarily as old (O), 39 were under 50 years and were classified as young (Y).

For all subjects, informed consent was obtained and health status was assessed based on medical records and physical examination. All Y subjects were in good health and did not take any medication. In the O group, 10 subjects did not suffer from any disease and did not take any medication while the others episodically took non-steroidal anti-inflammatory drugs and analgesics for chronic dorso-lumbar pain with minor impairment. All subjects had moderate and occasional physical activity.

Height and weight were measured for all individuals and were used to calculate the body mass index (BMI, ratio of weight (kg) to square height (m^2)). This index is considered a simple anthropometric feature of fatness [14].

2.1. ^{31}P MR spectroscopy

^{31}P MRS explorations of finger flexor muscles were carried out as previously described [15] using a Bruker 47/30 Biospec spectrometer interfaced with a 30 cm bore, 4.7 T horizontal superconducting magnet. Subjects sat on a chair by the magnet and positioned the flexor digitorum superficialis over a 50 mm diameter double-tuned surface coil. Pulsing conditions (1.8 s interpulse delay, 120 ms pulse length) were chosen to optimize the ^{31}P MR signal averaged each min (32 scans) during 3 min of rest, 3 min of exercise and 20 min of recovery. Exercise consisted in finger flexions performed at 1.5 s intervals, women lifting a 4 kg, men a 6 kg weight. All subjects were able to complete the exercise. The 3 min exercise duration and intensity were chosen to ensure that all subjects could complete the protocol and reached a significant decrease in pH and phosphocreatine concentration. The sliding of the weight was recorded using a home-built displacement transducer connected to a personal computer. Force was measured using ATS software (Sysma-France) and power output (W) was calculated at each minute of exercise.

2.2. Data analysis

Raw MR data were transferred to an IBM RISC 6000 workstation and processed using the NMR1 spectroscopy processing software (New Methods Research, Inc., Syracuse, NY, USA) as previously described [16]. Absolute concentrations of phosphocreatine (PCr), inorganic phosphate (Pi), adenosine triphosphate (ATP) and phosphomonoester (PME) were calculated after curve fitting of the spectrum to Lorentzian lines and a correction for differential magnetic saturation [15]. The corrected areas were expressed as absolute concentrations relative to ATP concentration measured at rest (8.2 mmol/l of intracellular water) [15]. The free cytosolic [ADP] was calculated from the creatine kinase equilibrium, assuming a total creatine content of 42.5 mM. Intracellular pH was calculated from the chemical shift of Pi relative to PCr [17]. Resynthesis of PCr during the post-exercise recovery period was fitted to a first-order exponential process as described by McCully et al. [18] and characterized by two parameters: the kinetic constant (k) of the process and the initial rate of PCr resynthesis.

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Table 1
Anthropometric data

	Men	Women	Young	Old
Number	30	26	29	27
Height (cm)	177 ± 1.1	161.6 ± 1.2*	175 ± 1.6	165 ± 1.7*
Weight (kg)	77 ± 1.5	60.2 ± 2*	70 ± 2.4	68 ± 2.4
BMI (kg/m ²)	24.5 ± 0.5	23 ± 0.8*	22.8 ± 0.6	24.9 ± 0.6*

Results are presented as means ± S.E.M.

* $P < 0.01$ (two-way ANOVA).

For each minute of exercise, rates of ATP production from PCr hydrolysis (P_{ATP}), glycogenolytic (G_{ATP}) and oxidative (O_{ATP}) pathways were calculated according to the method recently proposed by Kemp et al. [19–21]. The energy cost of contraction was defined as the amount of ATP utilized to produce a unit of power and represents the balance between rates of ATP consumption (by myosin ATPase and ionic pumps) and ATP production (by both anaerobic and aerobic pathways).

2.3. Statistical methods

Results are presented as means ± S.E.M. and P values less than 0.01 were considered significant. Two-way analysis of variance (ANOVA) and covariance (ANCOVA), with and without repeated measurements (time is the repeated parameter), were used to determine the effects of age, gender, and the age-gender interaction. When a statistically significant F value was obtained, a one-way ANOVA, using the pooled variance, was applied to examine the age effect in each gender. Regression analysis was performed to examine the association between variables of interest. The whole set of statistical analyses was performed with SAS software (SAS Institute Inc., Cary, NC, USA).

3. Results

Male subjects were taller and heavier than female subjects (Table 1). Young men and women were leaner than older subjects. The mean BMI in the four subgroups was normal while significant differences between groups were noted (Table 1).

3.1. At rest

There were no significant differences between males and females, nor between young and old subjects for metabolic parameters recorded at rest suggesting that metabolic status is not altered either by sex or by age (Table 2). Values of the mean buffering power of the muscle cytosol were in agreement with previously reported data [20] (Table 2). There were no differences among groups for the $[PCr]/[Pi]$ ratio.

3.2. During exercise

3.2.1. Power output. Men developed significantly larger power output during exercise (Fig. 1A). However, those differences disappeared when statistically adjusted by ANCOVA analysis for weight, height or BMI. Interestingly, height and weight were both linearly linked to power output ($r = 0.6$, $P < 0.001$ and $r = 0.4$, $P < 0.003$) whereas BMI and power output were not. Actually, when analyzed separately according to sex, BMI was inversely correlated to power output in women but not in men ($r = 0.59$, $P < 0.002$; Fig. 3).

During exercise, the rates of ATP production were first scaled to power output (P_{ATP} , G_{ATP} and O_{ATP}) then successively adjusted by ANCOVA analysis to weight, height and BMI. Adjustment of energy parameters for BMI was particularly indicated because of the sex dependence of BMI correlation to power output.

Table 2
MR metabolic parameters recorded during the rest-exercise-recovery protocol

	Men	Women	Young	Old
Number	30	26	29	27
<i>Rest</i>				
[PCr] (mM)	36.7 ± 0.7	36.3 ± 0.7	38.1 ± 0.4	36.6 ± 0.3
pH	6.9	7	7	7
[ADP] (mM)	8.6 ± 0.3	9.6 ± 0.5	9.9 ± 0.6	8.2 ± 0.3
[PCr]/[Pi]	11.7 ± 1.8	11 ± 0.8	11.6 ± 1.1	11 ± 1.1
Mean buffer (slykes)	31.3 ± 0.8	29.8 ± 0.9	29.2 ± 0.8	32.1 ± 0.9
<i>Differences between rest and end of exercise for PCr and pH</i>				
[PCr] (mM)	22.8 ± 1.4	17.5 ± 1.7*	22.7 ± 1.5	17.8 ± 1.6
pH	0.5	0.4*	0.4	0.5
<i>During exercise</i>				
Energy cost of contraction (EC) (mM/min/W)	22.3 ± 1.7	45 ± 3.6*	26.6 ± 3.2	39.4 ± 3.3
<i>End of exercise</i>				
[ADP] (mM)	23.4 ± 2.6	14.4 ± 2.2	22.3 ± 2.5	15.8 ± 2.4
Anaerobic ATP contribution (% of EC)	72.0 ± 2.4	62.5 ± 2.7	70.5 ± 2.7	64.6 ± 2.6
Aerobic ATP contribution (% of EC)	27.9 ± 2.4	37.4 ± 2.8	29.5 ± 2.7	35.4 ± 2.6
<i>Recovery</i>				
Proton efflux scaled to pH changes	6.6 ± 0.4	5.9 ± 0.5	6.3 ± 0.3	6.2 ± 0.5
Rate of PCr resynthesis (mM/min)	16.4 ± 2.5	19.15 ± 2.1	16.7 ± 1.5	18.7 ± 2.5

Results are presented as means ± S.E.M.

* $P < 0.01$ (two-way ANOVA and ANCOVA).

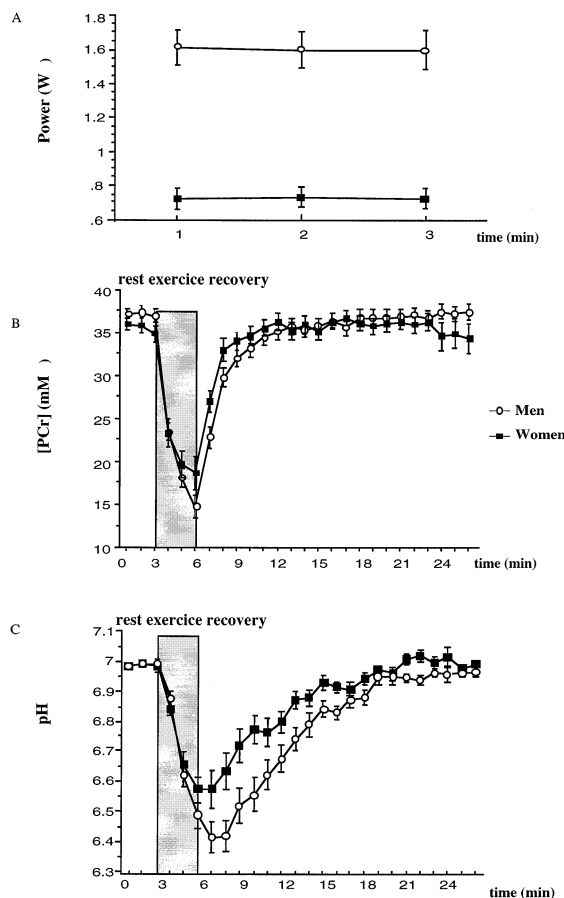


Fig. 1. Time-dependent evolution of power during exercise (A), concentration of PCr (B), and pH (C) throughout the rest-exercise-recovery protocol. The shaded areas correspond to the exercise period. Bars show the mean \pm S.E.M.

3.2.2. Metabolic changes. During exercise, [PCr] decreased as a result of PCr hydrolysis to maintain ATP homeostasis while a concomitant rise in P_i occurred. Women seemed to consume less PCr (Fig. 1B, Table 2). However, when taking into account power output, PCr consumption was larger in women than in men and remained larger after adjustment for BMI as shown by ANCOVA analysis. At the same time, a pH drop was noted as a sign of glycogenolytic ATP production. At the end of exercise, the extent of pH decrease calculated in men; adjusted for power output it was lower than in women. Adjustment for BMI did not modify this difference.

3.2.3. Quantitative analysis of ATP production. The quantitative analysis of ATP production during exercise was performed taking into account time-dependent changes of [PCr] and pH throughout the rest-exercise-recovery protocol [19–21]. The contributions of the different metabolic pathways to ATP synthesis scaled to power output are illustrated in Fig. 2.

3.2.3.1. ATP production from PCr scaled to power output (P_{ATP}). In all subjects, the rate of ATP production from PCr hydrolysis (P_{ATP}) decreased with respect to time as a result of increasing participation of glycogenolysis and oxidative metabolism (Fig. 2A). P_{ATP} was significantly higher in women (8.5 ± 1 vs. 5.1 ± 0.5 mM/min/W) at the onset of exercise, and similar for the remaining exercise period. Although the rate of ATP production seemed at first glance faster for

older subjects at the onset of exercise (8.2 ± 0.9 vs. 5.3 ± 0.5 mM/min/W), statistical significance was not achieved.

3.2.3.2. Glycogenolytic ATP production. During the exercise period, the maximum glycogenolytic rate of ATP production was noted either at the first minute (23.9 ± 2.8 mM/min/W) for women or at the second minute (18.9 ± 2.7 mM/min/W) for men (Fig. 2B). The lowest values were systematically measured at the end of exercise.

3.2.3.3. Energy cost. The energy cost (EC), calculated at the beginning of exercise considering that oxidative participation in ATP synthesis is negligible, was twice larger for women (45 ± 3.6 mM/min/W) as compared to men (22.2 ± 1.7 mM/min/W). Again, these values still differed when adjusted for weight and BMI. In other words, other things being equal, untrained women need more energy than untrained men to produce a similar level of power (normalization by power).

3.2.3.4. Oxidative ATP production. The rate of aerobic ATP production, calculated indirectly from EC and total anaerobic ATP production at each min of exercise, increased during exercise. The maximum value, reached at the end of the exercise period, was significantly larger in women (17.5 ± 2.4 vs. 6.7 ± 0.91 mM/min/W) as compared to men (Fig. 2C). When not scaled to power output, this rate (22.3 ± 3.1 vs. 18.1 ± 2.1 mM/min for the women as compared to men) is close to the initial rate of PCr recovery (Table 2), which is also considered a reasonable estimate of the end exercise rate of oxidative synthesis.

3.2.3.5. Relative contributions of each pathways. The relative contributions of each pathway scaled to energy cost were

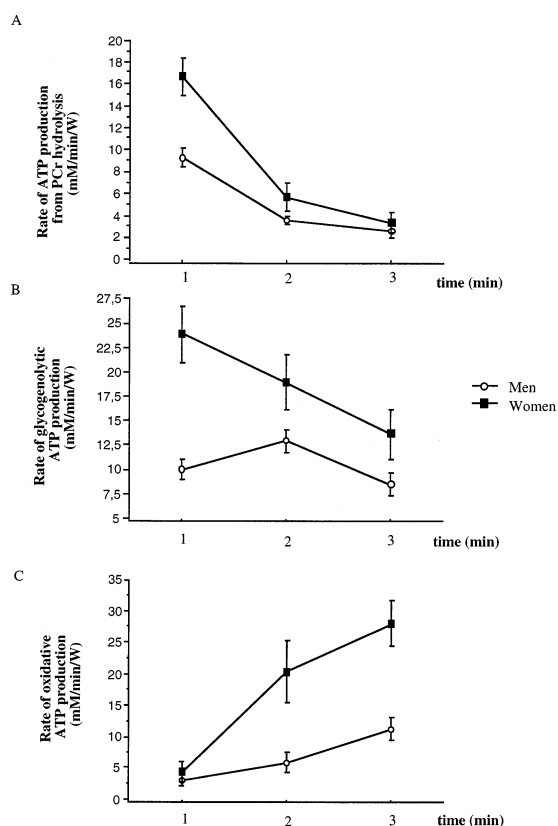


Fig. 2. Evolution of the rate of ATP production from PCr hydrolysis (A), glycogenolysis (B), and aerobic metabolism (C) during exercise in men and women. Results are presented as means \pm S.E.M.

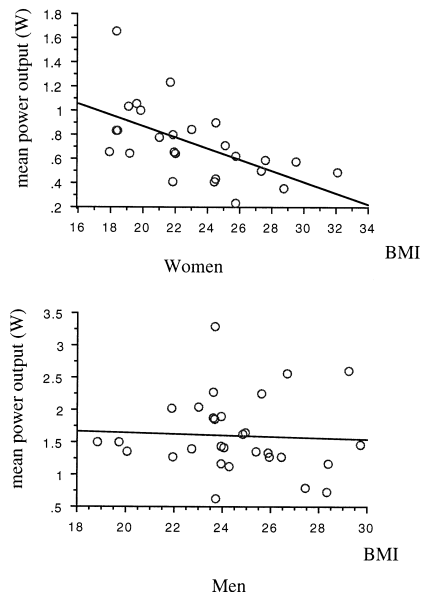


Fig. 3. Correlation between BMI and power output in men and women.

similar in the four groups thereby indicating no over- or underactivation of any pathway during exercise (Table 2).

Considering the whole set of parameters measured during exercise, a significant interaction was noted between sex and BMI suggesting that BMI affects those parameters differently in men and women. In contrast, the two-way analysis of variance for age and sex did not indicate any statistical differences between young and old subjects for the same set of parameters. Also, the statistical differences generated by gender were independent of age.

3.3. During recovery

At the end of exercise, PCr and pH both returned to rest values as a result of proton efflux and oxidative ATP synthesis. The initial rate of PCr resynthesis was similar in men and women suggesting that aerobic metabolism is not altered by gender. The kinetic constant of pH recovery calculated as the ratio of efflux adjusted to the extent of pH changes, measured at the end of exercise, was similar in the two groups (Fig. 1, Table 2). At the end of recovery, all metabolic parameters had reached their respective pre-exercise values.

4. Discussion

This ^{31}P MRS quantitative analysis indicates an increased energy cost for women as compared to men. Men displayed larger power output than women, but this difference disappeared after adjustment by statistical analysis for height, weight or BMI. Thus, the observed difference in power output can be easily explained by anthropometric factors. In contrast, the difference in energy cost of contraction was not suppressed by similar adjustment for weight and BMI, thereby suggesting a gender-related difference in muscle energetics. In other words, when taking into account equivalent BMI, men and women actually displayed the same power output, but women spent more energy to achieve it.

The energy cost can be increased by several mechanisms including augmented ATP consumption [16], pathological

changes in oxidative and/or glycogenolytic ATP contributions [22], alteration of effective muscle mass (the product of true muscle mass and contractile efficiency) [21], and/or a combination of any of those mechanisms. Increased ATP consumption activity has been related to the increased energy cost reported in hyperthyroidism [16]. Such a pathological increase in mechanisms of ATP utilization seems unlikely to occur in untrained healthy women since no evidence of gender-related hypermetabolism has ever been reported [23]. Anaerobic ATP production can be increased as a result of oxidative deficiency thereby leading to a higher energy cost [24]. Such a mechanism could be invoked since reduced maximal aerobic power and respiratory exchange ratio have been reported in women [23]. However, the analysis of PCr recovery kinetics, providing information on oxidative metabolism, and measurements of relative rates of aerobic ATP production did not indicate any alteration of oxidative metabolism in the group of untrained women. Also, biochemical analyses of biopsy specimens have demonstrated that the activities of oxidative enzymes are similar in men and women [1,12,13].

An increased rate of anaerobic ATP production could also directly result from a hyperactivation of glycogenolysis. However, calculations of relative rates of anaerobic ATP production did not support this hypothesis in agreement with biopsy studies, which have never suggested such an overactivation and have generally rather reported lower glycolytic enzyme activity in women [1,11,13,25]. In addition, glycogenolytic hyperactivation should have led to an abnormally large acidosis at each minute of exercise. This was not observed.

Overall, our results support the hypothesis that the higher level of ATP production observed in women is not related to an abnormal metabolism but rather to a decreased efficiency in ATP utilization. This in turn could result from an increase in the requirements for ATP due to reduced muscle mass and/or metabolic efficiency. Faced with a given work, a larger muscle mass would require less ATP as a result of increased ATP synthesis as previously suggested [26]. Muscle volume has been successfully used to normalize metabolic parameters recorded during exercise [27–29]. However, this normalization has been reported during moderate exercise without pH changes and presumes a direct relationship between muscle volume, the number of mitochondria and the supporting intermediary metabolism. In the present study, at least a two-fold reduction in activated muscle volume (which seems very unlikely) would be required to explain the increased energy cost measured in women. Besides muscle volume, gender differences in strength have also been corrected by adjustments for body weight [30], height or lean body weight [31]. The validity of these adjustments has been strengthened by the recent demonstration of height and weight as the principal determinants of appendicular skeletal muscle mass [32]. BMI is more an index of body fatness. We found that body fat mass was inversely correlated with power output in women. This correlation, which did not exist in men, indicates a larger influence of body fatness on muscle capacity in women. If an underestimation of total body fat by BMI in women had been made [14], the role of fatness on the observed reduced capacity would have been reinforced.

Besides a possible decreased muscle mass, a lower muscle efficiency (defined as the energy expenditure adjusted to mechanical power) might account for the increased energy cost of contraction. This lower efficiency could result from differ-

ences in proportion of muscle fibers between men and women. However, most human muscles are mixed and contain similar contingents of type I and II fibers [23]. If interindividual differences in fiber proportions do exist [1,7,13], they are minor and cannot explain large differences in muscle performance, at least in untrained subjects. Perhaps fiber size, which seems larger in men, plays a larger role in strength differences than does fiber proportion [4,11,33]. The lower efficiency could also result from differences in the metabolic potential of muscle fibers. Studies of enzyme activity on muscle biopsy specimens often indicate a more pronounced glycolytic and contractile potential in men as compared to women [1,7,11–13]. While still uncertain, these differences could reflect differences in intrinsic properties of muscle fibers. The increased energy cost observed in untrained women could then be explained, at least in part, by these disparities in metabolic potential.

Finally, the lower impact of age-induced metabolic alterations is not totally surprising considering the age of our subjects (most were under 70 years). Strength and endurance generally decrease with age but strength decline is most dramatic after the age of 70 [6,34]. Previous MRS studies on age are controversial, reporting the existence of a relationship [18,35] or no relationship [36] between biochemical and histological changes observed *in vitro* and muscle energetics. In the current study, variations in age did not alter gender-related differences.

In conclusion, *in vivo* MRS shows that untrained women display a higher ATP cost of contraction during muscle exercise, compensated by activation of both anaerobic and aerobic energy-producing pathways. It also shows that gender differences have more impact on muscle skeletal metabolism than age differences, given an adult range of 20–75 years. Alterations of anaerobic and/or aerobic contributions to ATP production have been ruled out as possible causes of the increased energy cost. The increase in muscle energy cost might be explained partly by differences in local muscle mass, a higher impact of fatness, but also by a reduced metabolic efficiency of muscle fibers in untrained women.

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